METHODS OF INHIBITION OF PROTEIN FUCOSYLATION IN VIVO USING FUCOSE ANALOGS

CROSS-REFERENCES TO RELATED APPLICATIONS

[0001] This application is a continuation of U.S. application Ser. No. 15/299,894 filed on Oct. 21, 2016, which is a continuation of U.S. application Ser. No. 13/814,083 filed on Feb. 4, 2013, which is the national stage filing under 35 U.S.C. § 371 of International Application No. PCT/US2011/046857 filed Aug. 5, 2011, which claims the benefit of U.S. Provisional Application No. 61/371,116, filed Aug. 5, 2010, the disclosures of each are incorporated by reference herein in their entirety.

BACKGROUND OF THE INVENTION

[0002] L-fucose, also referred to as 6-deoxy-L-galactose, is a monosaccharide that is a component of some N- and O-linked glycans and glycolipids in animals. (See Becker and Lowe, Glycobiology 13:41R-51R (2003).) Fucose is typically added as a terminal modification to glycans, including glycans attached to blood group antigens, selectins and antibodies. Fucose can be attached to glycans via α(1,2)-, α(1,3)-, α(1,4)- and α(1,6)-linkages by specific fucosyltransferases. α(1,2)-fucose linkages are typically associated with the H-blood group antigens. α(1,3)- and α(1,4)-fucose linkages are associated with modification of $Lewis^X$ antigens. α(1,6)-fucose linkages are associated with N-linked GlcNAc molecules, such as those on antibodies.

[0003] Fucosylation of proteins is believed to play a role in mammalian development. Mice homozygous for a targeted mutation of the FX gene exhibit pleiotropic abnormalities including a lethal phenotype. Reduced recovery of mice from heterozygous crosses was also reported. (Becker et al., Mammalian Genome 14:130-139 (2003). Aberrant protein fucosylation has been proposed to be associated with human disease, including up-regulation of sialyl $Lewis^X$ and sialyl $Lewis^y$ in cancers. These glycans are ligands for E- and P-selectin molecules. In it speculated that increases in sialyl $Lewis^X$ and sialyl $Lewis^y$ glycans on cancer cells increases metastases through interaction with E- and P-selectins on endothelium. Increased fucosylated glycans have also been observed in patients with rheumatoid arthritis. Currently, however, there are no approved therapeutic approaches targeting protein fucosylation levels.

SUMMARY OF THE INVENTION

[0004] The methods and compositions described herein are premised in part on the unexpected results presented in the Examples, showing that animals administered a fucose analog have reduced protein fucosylation. Fucosylation of antibodies and other proteins can be modulated using the fucose analogs described herein.

[0005] In one aspect, methods and compositions for the in vivo production of defucosylated proteins are provided. Animals, such as mammals, administered a fucose analog (having formula I, II, III, IV, V or VI) produce proteins, such as cell surface proteins, having reduced fucosylation. The reduction in fucosylation is relative to animals untreated with the fucose analogs having formula I, II, III, IV, V or VI, respectively.

[0006] In a related aspect, methods and compositions for the in vivo production of antibodies and antibody derivatives with reduced core fucosylation are provided. Animals administered a fucose analog (having formula I, II, III, IV, V or VI) produce antibodies and antibody derivatives having reduced core fucosylation (i.e., reduced fucosylation of N-acetylglucosamine of the complex N-glycoside-linked sugar chains bound to the Fc region through the N-acetylglucosamine of the reducing terminal of the sugar chains). The reduction in core fucosylation is relative to animals untreated with the fucose analogs of having formula I, II, III, IV, V or VI, respectively.

[0007] In another aspect, pharmaceutical compositions containing fucose analogs and formulated for administration to a target animal are provided. The fucose analogs can be formulated for administration to an animal to inhibit or reduce fucosylation in vivo.

[0008] These and other aspects of the present invention may be more fully understood by reference to the following detailed description, non-limiting examples of specific embodiments, and the appended figures.

BRIEF DESCRIPTION OF THE DRAWINGS

[0009] FIG. **1** shows the results of administration of fucose analogs (via ip injection) on antibody fucosylation. Dot blots are shown on the left panel and a graph is shown on the right panel. The dot blot protein loading levels (upper left) and fucose-specific bioluminescence (lower left) for antibody cAC10 standards (lower dot blot, left most dashed rectangle and corresponding columns of upper dot blot), untreated control (lower dot blot, second dashed rectangle from the left and corresponding column of upper dot blot), and alkynyl fucose (SGD-1887; lower dot blot, middle dashed rectangle and corresponding column of upper dot blot), alkynyl fucose peracetate (SCD-1890; lower dot, blot, second dashed rectangle from the right and corresponding column of upper dot blot), and 2-fluorofucose (SGD-2083; lower dot blot, right most rectangle and corresponding column of upper dot blot). After correcting for loading level, the % fucosylation is shown on the graph at the right.

[0010] FIG. **2** shows the effects on antibody core fucosylation of administration of fucose analogs via drinking water. The graphs show % fucosylation of antibodies as a determined by gas chromatograph (GC): panels A and B show fucosylation levels of the anti-KLH antibodies (Abs) isolated from the treated groups while panels C and D show the fucosylation levels of the remaining (non-KLH-specific) IgG antibodies. Panels A and C show the percent fucosylation of each animal determined using a purified antibody standard curve (0-100% fucosylation). Panels B and D show the fucosylation level of the treated groups as a percentage of the average untreated control group value.

[0011] FIG. **3** shows the effects on antibody core fucosylation of administration of fucose analogs via drinking water. In this figure, fucosylation levels of the non-KLH-specific antibodies are shown. Dot blots of protein loading levels (upper left) and fucose specific bioluminescence (lower left) are shown for cAC10 standards (upper and lower dot blots, left most rectangle), untreated control (upper and lower dot blots, second from the left (upper) and right rectangles), and 2-fluorofucose (upper and lower dot blots, second from the left (lower) and second from the right rectangles (upper and lower)). After correcting for loading level, the % fucosylation is shown in the graph on the right.